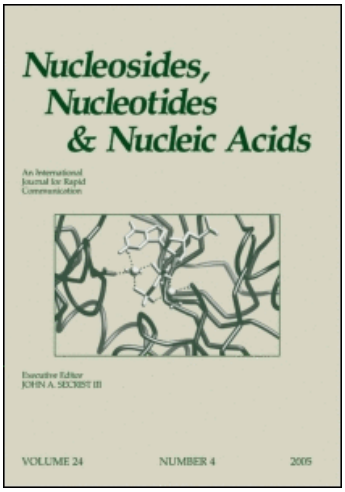

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PURIFICATION OF PMPA AMIDATE PRODRUGS BY SMB CHROMATOGRAPHY AND X-RAY CRYSTALLOGRAPHY OF THE DIASTEREOMERICALLY PURE GS-7340

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Gilead Sciences Inc., Foster City, California

ABSTRACT

The diastereomers of GS-7171, aryl phosphoramidate derivatives of the anti-HIV nucleotide analog 9-[2-*R*-(phosphonomethoxy)propyl]adenine (tenofovir, PMPA), were isolated by batch elution chromatography and continuous simulated moving bed chromatography. The absolute configuration of the more pharmacologically active diastereomer, GS-7340, was determined to be (*R*,*S*,*S*) by single crystal x-ray crystallography.

BACKGROUND

A series of aryl phosphoramidate derivatives of the anti-HIV nucleotide analog tenofovir were prepared as intracellar prodrugs. One of the analogs, GS-7171 (Fig. 1), was selected as a candidate for further evaluation on the basis of its potent *in vitro* anti-HIV activity and good oral bio-availability in dogs.

GS-7171 contains three chiral centers. The 2-propyloxy center of tenofovir (*R*), and the alanine-amidate (*S*) chiral center, are controlled synthetically (1). The stereochemistry at the phosphorus atom is not controlled with the current synthetic process, therefore, GS-7171 is synthesized as a 1:1 diastereomeric mixture, GS-7339 (Fig. 2) and GS-7340 (Fig. 3). FDA guidelines (2) recommend that

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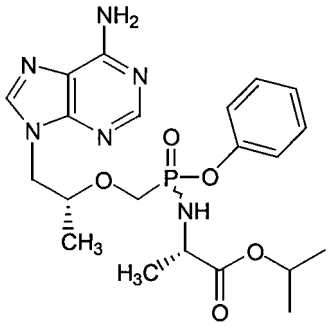

Figure 1. GS-7171.

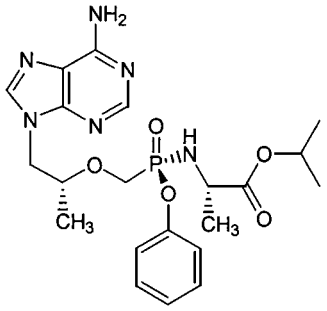

Figure 2. GS-7339.

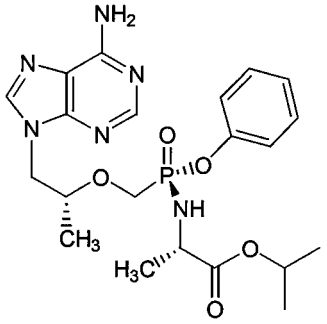

Figure 3. GS-7340.

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stereoisomers are resolved and evaluated individually for their therapeutic and toxicity profiles, and that where practical only a single stereoisomer be developed as an investigational drug. Hence, technology was needed to isolate the diastereomers of GS-7171 in gram quantities for biological and toxicological evaluation.

RESULTS AND DISCUSSION

Chromatographic theory predicts that achiral stationary supports can resolve diastereomers (3). However, initial screening studies yielded poor success for GS-7171 on reversed phase packings (Fig. 4). A commercially available chiral stationary phase (CSP), Chiralpak phase packings (Fig. 4). A commercially available chiral stationary phase (CSP), Chiralpak AS (amylose tris[(*S*)-α-methylbenzylcarbamate] on silica gel, Chiral Technologies, Inc., Exton, PA) proved to be uniquely suited for GS-7171 among the CSPs screened. The diastereomers were resolved with a large separation factor ($\alpha > 7$), in either of the two eluent systems evaluated, acetonitrile:2-propanol (90:10) (Fig. 5) or acetonitrile:methanol (75:25) (Fig. 6). The high solubility of GS-7171 in these eluents (>300 mg/mL) allowed very high column loading with minimal injection volumes.

Gram quantities of the resolved diastereomers were required to support *in vitro* anti-HIV testing and *in vivo* bio-availability studies. We employed batch elution chromatography (BEC) with a 20 μm, 21 $\times$ 250 mm, semi-preparative column, using the acetonitrile:2-propanol mobile phase system described above, to resolve a total of 2.5 grams of GS-7339 and GS-7340. Biological testing demonstrated that GS-7340 has a 10 fold greater anti-HIV activity ($IC_{50} = 0.005$ μM) than GS-7339 ($IC_{50} = 0.06$ μM) (4). On this basis GS-7340 was selected as a potential clinical candidate.

The projected GS-7340 material requirements to support clinical evaluation were set at slightly over 1 kg, this meant resolving about 2.5 kg of GS-7171.

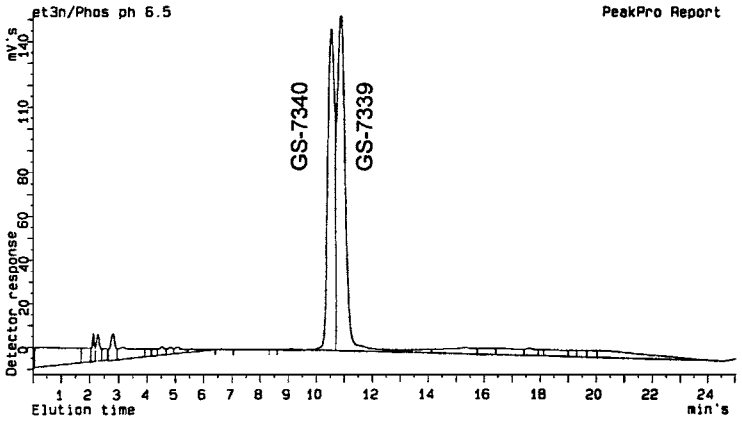

Figure 4. GS-7171 on C18, phosphate buffer:MeCN (gradient).

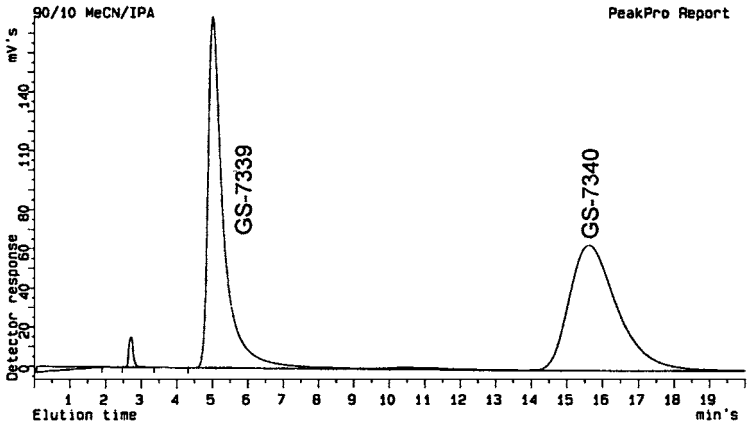

Figure 5. GS-7171, MeCN:2-Propanol (90:10).

The largest BEC system available was inadequate at this scale. Continuous Simulated Moving Bed (SMB) chromatography has been demonstrated to be effective for difficult chromatographic resolutions with high product throughput (5). The BEC conditions were quickly adapted for use in SMB operations, and 2.5 kg of GS-7171 was resolved on a pilot scale SMB system, with 10, 50 × 100 mm Chiralpak AS CSP columns.

The bulk of the recovered GS-7340, d.e. = 96.8% (Fig. 7) was converted to the fumaric acid salt, to support potential phase I clinical trials and for other preclinical activities. A portion of the GS-7340 recovered from SMB chromatography were crystallized from water as the free base, and submitted for single crystal x-ray crystallography. The x-ray crystal structure, demonstrated that the absolute configuration of the phosphorus chiral center was *S*.

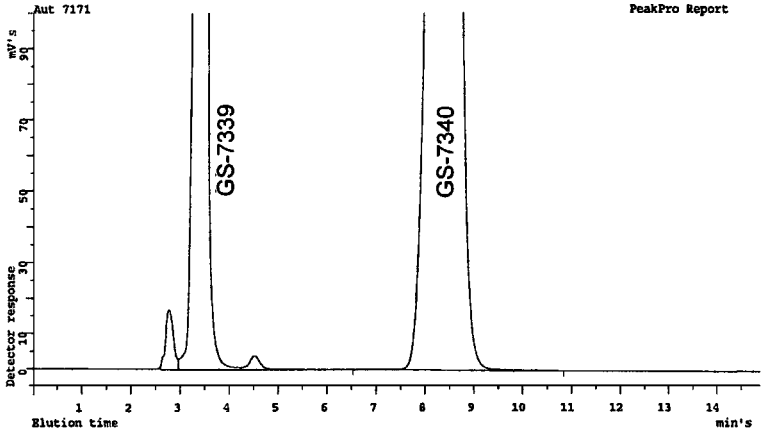

Figure 6. GS-7171, MeCN:MeOH (75:25).

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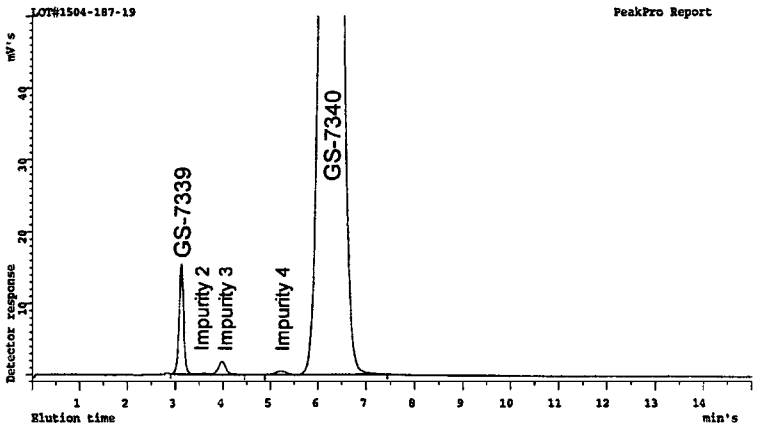

Figure 7. GS-7340 Fumarate, ex SMB, d.e. = 96.8%.

SUMMARY

Multi-gram quantities of GS-7339 and GS-7340, sufficient to conduct biological evaluation, were isolated from the diastereomeric mixture, GS-7171, by batch elution chromatography on Chiralpak AS stationary phase. GS-7340 was shown to be about 10 fold more potent against HIV than GS-7339, $IC_{50} = 0.005\ \mu M$ versus 0.06 μM. A multi-kilogram process for the purification of GS-7340 was developed using SMB chromatography, allowing sufficient quantity (1.3 kg) and purity (d.e. = 96.8%) to support further development. The material isolated by SMB chromatography also enabled the isolation of crystals of GS-7340 free base. The absolute configuration of the phosphorus chiral center was determined by x-ray crystallography to be *S*.

ACKNOWLEDGMENTS

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